

THERAPEUTIC COMBINATION

[0001] The present invention relates to combinations of agents which are useful in the protection of normal cells during cancer treatment with a cytotoxic agent, to kits of pharmaceutical compositions comprising these, and methods of treatment and dosage regimes which utilise these combinations.

BACKGROUND OF THE INVENTION

[0002] The presence of an origin activation checkpoint which arrests cells in G1 in response to perturbations in DNA replication initiation is supported by experimental evidence from several studies. This checkpoint in the DNA licensing machinery or DNA replication initiation machinery can be induced using many mechanisms including RNAi against Cdc7, an essential kinase involved in the initiation of DNA synthesis at licensed chromosomal replication origins through phosphorylation and activation of the Mcm2-7 helicase. Other targets for disruption of the checkpoint have been found to be ORC1-6, Cdc6, Cdt1, geminin, Dbf4 Cdc45, GINS, Pol ϵ , Mcm10, Sid3, Sid5, Sid7, Sid2, Dpb11, Pol α , Ctf4, PCNA, Pfs1, Pfs2 and Psf3.

[0003] In normal cells, the checkpoint prevents entry into a lethal S phase in the presence of an insufficient number of replication competent origins. In contrast, many cancer cells have a defective checkpoint, which leads to fork stalling/collapse, an abortive S phase and apoptotic cell death.

[0004] The molecular architecture of the origin activation checkpoint has recently been characterised and it has been shown that in normal cells arrest of cells in G1 phase can be reversed. (Rodriguez-Acebes S, et al. *Am J Pathol* 2010; 177:2034-45; PMID: 20724597; DOI:10.2353/ajpath.2010.100421; Tudzarova S, et al. *EMBO J* 2010; 29:3381-94; PMID: 20729811; DOI: 10.1038/emboj.2010.201). The checkpoint response in arrested cells was shown to be dependent on 3 non-redundant axes mediated by FoxO3a, involving upregulation of CDK inhibitor p15INK4B, activation of the p14ARF-MDM2-p53-p21 pathway, and p53 mediated upregulation of the Wnt/-catenin pathway antagonist DKK3, which leads to Myc and cyclin D1 downregulation. The resulting loss of CDK activity inactivates the Rb-E2F pathway, overrides the G1-S transcriptional program and leads to a robust G1 cell cycle arrest.

[0005] The involvement of several tumour suppressor genes (TSGs) frequently inactivated during tumorigenesis and the lack of redundancy may account for why cancer cells have a defective origin activation checkpoint arrest. Furthermore, this provides a mechanistic basis for the cancer-cell-specific killing observed with emerging pharmacological Cdc7 inhibitors (Montagnoli A. et al., *Nat Chem Biol* 2008; 4:357-65; PMID: 18469809; DOI: 10.1038/nchembio).

[0006] Anti-mitotic chemotherapeutic agents remain a cornerstone of multimodality treatment for locally advanced and metastatic cancers. For example, the potent anti-mitotic taxane, paclitaxel, is broadly used in neoadjuvant/adjuvant therapy and also in the treatment of metastatic disease. A drawback of these current chemotherapy regimens, however, remains the associated toxicity in normal tissues with high cellular turnover, for example of the bone marrow, hair follicle cells, and gastrointestinal tract epithelium. This often leads to undesired side effects such as myelosuppression (e.g. neutropenia), hair loss and gastrointestinal toxicity, and consequently dose reduction and incomplete administration

of prescribed regimens, allowing survival of tumor cells and the development of drug resistance.

[0007] Therefore novel approaches to enhance the therapeutic window of existing cytotoxic chemotherapies are required.

[0008] Cyclotherapy is a strategy aimed at exploiting differences between normal and cancer cells to selectively protect normal proliferating cells from the cytotoxic effects of chemotherapy, thereby increasing the therapeutic window (Blagosklonny M V et al. *Cell Cycle* 2001, 1:375-82; PMID: 12548008; DOI: 10.4161/cc.1.6.259; Blagosklonny M V et al. *Cancer Research* 2001, 4301-4305). This is based on the concept that as most cytotoxic chemotherapies preferentially target cycling cells, by selectively inducing a reversible cell cycle arrest in normal cells, these cells would thus be protected from cytotoxicity, and can re-enter the cell cycle unharmed. In contrast, cancer cells which are characterized by uncontrolled proliferation as the result of multiple genetic aberrations and loss of checkpoint regulation fail to cell cycle arrest and remain sensitive to cytotoxic chemotherapy. In summary, triggering the DNA replication initiation checkpoint in normal cells induces a reversible G1 arrest which therefore protects these cells from S phase and G2/M phase directed chemotherapeutic agents.

[0009] Many components of the DNA replication machinery/pathway have been proposed as anti-cancer drug targets. Cdc7 kinase in particular has been identified as an important target. Inhibition of DNA origin firing by targeting Cdc7 kinase with ATP-competitive SMIs or RNAi results in cancer cells entering an abortive S phase followed by apoptotic cell death. It has been shown (Mulvey et al. *Journal of Proteome Research* 2010, 9, 5445-5460) that normal somatic cells avoid entering a lethal S phase by engaging a DNA origin activation checkpoint that reversibly arrests cells in G1 phase, as illustrated schematically in FIG. 6. As a result, therapies involving such inhibitors would provide a specific and selective anti-tumour effect, which left normal cells undamaged, thus reducing unwanted side effects.

[0010] The pharmaceutical industry have selected in particular Cdc7 kinase and the MCMs as potential therapeutic targets and there are many drug development programmes in place throughout the world developing anti-cancer agents targeting these DNA replication licensing/initiation proteins. In general, this has focused on the generation of small molecule compounds but targeting of factors such as Cdc7 kinase could also be achieved using a range of biological agents such as RNAi, inhibitory peptides or immunoglobulins such as monoclonal antibodies or binding fragments thereof.

SUMMARY OF THE INVENTION

[0011] According to the present invention there is provided a combination of i) an inhibition or disruption agent which inhibits or disrupts the DNA licensing machinery and/or the DNA replication initiation machinery and ii) a cytotoxic agent which acts in either the G2, M and/or S phases of a cell cycle for use in the shielding of normal cells during cancer treatment, wherein the inhibition or disruption agent is administered to the patient first in an amount sufficient to reversibly arrest normal cells in G1 phase, and the cytotoxic agent ii) is administered subsequently.

[0012] The inhibition or disruption agent which inhibits or disrupts the DNA licensing machinery and/or the DNA